RELATION OF VIABILITY TO GLUTAMIC AND PYRUVIC DECARBOXYLASES IN WHEAT (TRITICUM VULGARE)

by

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INTRODUCTION

The importance of cereal grains in the growth of civilization has been due to a large extent to their good keeping qualities when stored under ideal conditions. However, unfavorable weather during harvesting and improper storage initiate various deteriorative processes in the grain. All these deteriorative changes lower the value of cereal grain both for milling processing and its use for seed. Hence an understanding of the scientific principles of grain storage is of great economic importance.

In the present study various factors involved in deterioration of wheat grains have been investigated with a special attention to biochemical methods in determining the degree of viability. Preliminary evidence from this laboratory indicated a possible correlation between viability and decarboxylation of glutamate (15). Hence the relation of the type of damage to the particular method involved was studied with special emphasis on the relationship of "sick" wheat to the application of glutamic and pyruvic acid decarboxylases in viability determinations.

REVIEW OF THE LITERATURE

Factors Involved in the Loss of Viability of Cereal Grain

Grain damage can be divided into two main categories, namely field damage and storage damage. Field damage, such as weathering, fungal, sprout, frost, etc. damage is generally of lesser economic importance than types of storage deterioration.

Tuite and Christensen (101) have shown in wheat that very little mold infection takes place in the head of wheat in the field or in freshly threshed wheat, but infection occurs to a great extent in the country elevators and

still more in the terminal elevators. The same authors (100) found that this also is true of malting barley. Fungal mycelium is commonly found under the pericarp of wheats grown throughout the world (45), but this internal mycelium appears largely to be that of Alternaria and other fungi which do not grow at the moisture levels ordinarily encountered in storage (18, 19, 20, 45).

It has been known for a long time that the most important environmental factors affecting the viability and storage life of grains are temperature and humidity (4, 38, 76, 108). The increase in temperature and moisture are followed by a gradual increase in respiration, as well as by the development of common storage molds such as Aspergillus sp. and Penicillum sp. Aspergillus sp. among other fungi has been thought to be the major contributing factor in the development of germ-damaged or so-called "sick" wheat in stored grain (13, 18, 19, 21, 76), and in various biochemical changes that accompany such deterioration (13, 43). Recent findings indicate, however, that metabolic reactions involving embryo itself, may play an important role in the storage life of cereal grains (17, 60, 61, 62, 66).

Methods for Determining Viability

Germination test. The classical germination test on wet filter paper or on sterilized, wet sand is still recognized as the best and most objective method for determination of viability and vigor of seeds.

In seed testing, germination is defined as the emergence and development from the seed embryo of those essential structures which, for the seed in question are indicative of the embryo's ability to produce a normal plant under favorable conditions (47). Several factors have to be considered while judging the results from a germination test. Dormancies of seeds are biologically significant in delaying germination until the environment is favorable for

development of the seedlings (40). Some seeds will only germinate after a prolonged period of dry storage; some only after exposure to subfreezing temperatures. Certain conditions must universally be met for germination, such as moisture for rehydration, temperature within a suitable range, and oxygen for respiration. Basic work (29, 42, 67, 83) has shown that the germination process is controlled by several interacting factors which have to be in a proper balance before germination can begin.

Biochemical tests. The need for a quick and reliable test for seed quality and viability has been understood for many years. In 1922 Turesson (102) introduced a method using the enzymatic reduction of methylen blue as an indicator for viability. Seven years later (77) the Russians used successfully indigo carmine for testing quick germinating seeds. Eidmann (30), and Gadd and Kjaer (35) used selenium salts as germination indicators.

Lakon suggested that death of the seed is a gradual process, so that certain parts of the embryo would succumb before others. He first employed selenium salts (53, 54) to distinguish between living and non-living portions of the embryos of small grains. Depending on staining of a seed, he was able to determine whether it was capable to germinate or not. Later he tested several tetrazolium salts in this respect, finding 2,3,5-triphenyl tetrazolium chloride most suitable (55). The method is based on the enzymatic reduction by living cells of colorless tetrazolium chloride to formazan, an insoluble red dye. Kuhn and Jerchel (51) confirmed Lakon's results. Working with corn and small grains, Lakon concluded (55, 56), although backed by a relatively small amount of data, that an accurate estimate of potential viability of seeds could be made within 8-24 hours of time. Seeds were soaked in water overnight, bisected longitudinally and transferred into the tetrazolium solution. After 4-24 hours, living parts of the embryo stained bright carmine red due to the

formation of formazan. He (57) favored the tetrazolium test over the germination test due to its speed. Porter, Durrell and Romm (77) applied Lakon's method to a number of common agricultural seeds as: cereals, corn, peas, soybeans, and sorghum. They were able to obtain a fair correlation between staining and germination tests for members of the Graminae. Cottrell (24, 25) modified slightly Lakon's method, obtaining satisfactory results for various seeds of the grass family. He (25) concluded that biochemical methods were useful for special purposes, but should not replace the germination test as suggested by Lakon (57, 58). Ritvanen (82) applied tetrazolium chloride to determination of the viability of timothy.

Shuel (88) carried out tests with oats, barley, and wheat, attempting to eliminate the necessity for hulling the seeds. His procedure consisted of immersing entire grains in a one percent solution of tetrazolium chloride. Evaluation of staining was done by external observation of the embryo. Wheat seeds with the scutellum only partially colored were judged non-viable. Staining of unhulled oats was observed by holding seeds in front of an electric light bulb.

Smith and Thorneberry (90), while investigating the mechanism of the tetrazolium reaction in seeds, felt that the dehydrogenases were probably responsible for the reduction of tetrazolium, and they presented a detailed reaction sequence through which malic dehydrogenase, or possibly other dehydrogenases might carry out this reduction. Working with corn and oats, Thorneberry (99) and Smith (89) confirmed the dehydrogenase hypothesis. Since the dehydrogenases constitute a delicate and complex system vital in respiratory activity, it might be presumed that an accurate assay of dehydrogenase activity would be a good measure of viability. The tetrazolium salt is thus an oxidation-reduction indicator, and the development of the non-diffusible red color in a

specific tissue is presumably an indication of the presence of active respiratory processes.

Other enzymes than dehydrogenases have been also studied in relation to viability of seeds. Activity of enzymes hydrolyzing carbohydrates, fat, and proteins is often significant for the early stages of germination. Mar (65) showed that amylase activity of soaked oat seeds is definitely correlated with germinability. French (33) investigated the ability of the excised embryo to form starch or amylase as an index of viability and seedling vigor in barley. He found that the number of embryos giving a positive test for starch with iodine correlated closely with the germination percentage in heat damaged barley. Crocker and Harrington (26), Davis (27), and Leggatt (59) have published evidence indicating that catalase activity could be used as a measure of viability. Leggatt (59) showed that the correlation between germinability and the ratio of total and thermostable catalase was highly positive.

Glutamic and Pyruvic Acid Decarboxylases in Higher Plants

Glutamic Acid Decarboxylase. Amino acid decarboxylases, which in one step break down amino acids into carbon dioxide and an amine, were first described by Gale (36) in microorganisms. Since that time a multitude of amino acid decarboxylases have been detected (68).

Glutamic acid decarboxylase is by far the most predominant amino acid decarboxylase in higher plants. In 1913 Abderhalden, et al. (1) found that microorganisms were able to form gamma-amino butyric acid directly from glutamate. The enzyme was first described in higher plants by Okunuki (75) in 1937. Schales et al. (85, 86, 87) reported the presence of glutamic acid decarboxylase in 30 different plants, and it has been later reported by others in barley (10, 72), alder (93), legumes (52), and Scopolia japonica (97). The enzyme has

also been shown in brain (78, 106), in chlorella (104, 105), and in several microorganisms (11, 28, 50, 71). The reaction product gamma-aminobutyric acid was first identified among the naturally occurring free amino acids in potato tuber by Steward et al. (94), but very slight glutamic acid decarboxylase activity was found in these tissues (41, 93, 94, 98). Later gamma-aminobutyric acid has been found to be widely present in higher plants, but its metabolic fate is still largely unknown (64, 92).

Recently (17, 62, 79, 80, 81) glutamic acid decarboxylase and gamma-aminobutyric acid have been described in wheat. According to Linko and Milner (61) the enzyme is readily activated by addition of water to dry embryos, resulting in a marked decrease in free glutamic acid and an increase in gamma-aminobutyric acid. Previous work from this laboratory has shown (15) that glutamic acid decarboxylase rapidly disappears under unfavorable storage conditions, and its use for viability determination was suggested (16).

Pyruvic acid decarboxylase (carboxylase). In 1910, Neubauer (73) concluded that pyruvic acid is an intermediate product in alcoholic fermentation by yeast. He further suggested that pyruvic acid is decarboxylated to acetaldehyde, which is then reduced to ethyl alcohol. Later Neuberg and Karczag (74) confirmed this theory by showing that yeast possesses an enzyme, carboxylase, which decarboxylates pyruvic acid. Washed yeast cells lose the capacity to perform this reaction since the coenzyme is washed out (2, 3). This cocarboxylase has been later isolated and identified as thiamine pyrophosphate.

According to Convay and Mach Donnel (23) carbon dioxide is not directly split off when carboxylase acts upon pyruvic acid or other alpha-keto acids. They claimed that carbonic acid would be the primary product in contrast to the reaction in which urea is directly decomposed to carbon dioxide by urease. Krebs and Raughton (49), however, later showed that carbon dioxide instead of

H_2CO_3 or HCO_3^- is the primary product of enzymatic decarboxylation of alpha-keto acids. Carboxylase is widely present in living cells and is readily activated in wheat embryos upon wetting (15, 63).

MATERIALS AND METHODS

Materials

Two series of wheat samples were employed in this study. All of the 25 samples in the first series were commercial mixtures of hard red winter wheat, containing from 20 to 100 percent of germ-damaged, so-called "sick" kernels. The wheats were obtained from the Grain Division, Agricultural Marketing Service, USDA, Kansas City, Mo., and were stored in a cold room at +4°C for about one year. The moisture content of the samples varied from 9.35 to 12.91 percent. The second series consisted of samples shown in Table 1.

When whole grains were used they were ground with Waring Blendor for two minutes. For experiments with wheat germ, the germ ends of grains were carefully cut off by a razor blade so that no germ was left in the endosperm end. Small amounts of endosperm attached to germ end could be neglected due to the fact that practically all glutamic acid decarboxylase activity is located in the embryo (61). Isolated germ ends were ground with a mortar prior to the experiment.

Moisture Content

The grains were ground with the Waring Blendor for 2 minutes. The moisture content was determined by the one stage air oven method, drying samples for one hour at 130°C (14). All samples were stored in air-tight flasks at room temperature during the time the tests were carried out.

Table 1. Samples of wheat in series 2. (Moisture percentages are given in parentheses)

Sample No.	Type of Wheat		Source
1	HRS, Lightly frozen	(11.58%)	Grain Research Lab., Winnipeg
2	" Moderately frozen	(11.54%)	
3	" Badly frozen	(11.25%)	
4	" Very badly frozen	(11.20%)	
5	" Low protein content	(12.23%)	Cargill, Inc., Minneapolis
6	" Low protein content	(11.50%)	
7	" High protein content	(10.81%)	
8	" High protein content	(10.88%)	
9	" Sprout damaged	(12.42%)	
10	" Sprout damaged	(12.50%)	
11	" Low test weight	(11.25%)	
12	" Low test weight	(11.56%)	
13	HRW, High protein content	(11.61%)	Cargill, Inc., Omaha
14	" High protein content	(11.34%)	
15	" Medium protein content	(12.30%)	
16	" Medium protein content	(11.95%)	
17	" Low protein content	(11.42%)	
18	" Low protein content	(11.96%)	
19	" Low test weight	(11.88%)	
20	" Low test weight	(12.24%)	
21	" 78% germination	(11.57%)	Seed Laboratory Topeka
22	" 90% germination	(11.93%)	
23	" 98% germination	(11.36%)	
24	" 85% germination	(11.42%)	

"Sick" Wheat

"Sick" wheat was determined by carefully removing the pericap covering the germ. Germ-damaged wheat is recognized by a brownish discoloration of the embryo. If the embryo was cream to white colored it was considered alive and the wheat sound. In all other cases wheat was judged "sick". In general, there was good agreement between the duplicate determinations.

Germination Test

Grains were surface sterilized in a 0.1 percent $HgCl_2$ solution for two minutes, and rinsed five times in tap water before they were laid out for germination. Samples of 100 kernels each were placed crease down on moist sterile quartz-sand in petri dishes, covered with wet filterpaper and stored in a dark drawer at 24-26°C. Germinated grains were removed every second day. All kernels which showed normal sprouts after seven days were considered viable. Four replications for the first and two for the second series of samples were used.

Manometric Techniques

Assays were carried out in Warburg flasks according to the direct method outlined by Umbreit et al. (103). The method is based on the principle that at constant temperature and gas volume, any changes in the amount of gas can be measured by changes in its pressure. The apparatus consists of a removable flask equipped with one or more sidearms, attached to a manometer containing a liquid of known density. One sidearm vessels were employed. Ethyl lactate colored with crystal violet was used as manometer liquid. The reaction chamber of clean dry Warburg vessels received the enzyme preparation (usually 500 mg of ground wheat grains, or 100 mg of ground wheat germ). One milliliter of

standard 0.067 M (M/15) phosphate buffer of pH 5.8 (103) was pipetted into the sidearm. One flask-manometer combination was left without enzyme preparation in order to detect slight pressure and temperature changes independent from the enzyme activity. In addition to this so-called thermobarometer a typical experimental setup consisted of five pairs of flask-manometer combinations. One flask of a pair had a folded filter paper square soaked in 0.2 ml of 20 percent potassium hydroxide in the center well in order to absorb carbon dioxide evolved. This was necessary to correct the results for the oxygen uptake. The conditions in both flasks thus can be considered practically equal with the exception that in one flask the tissue respires in the presence of carbon dioxide, and in the other one in its absence. Generally, however, carbon dioxide has little effect on respiration.

After connecting the flasks to the appropriate manometers they were immersed in a water bath of 30.0 $\pm$ 0.1°C. After a ten minutes incubation period with stopcocks open the contents of the sidearms were tipped into the reaction chamber, and the shaker was started (106 to 108 strokes per minute). The stopcocks were left open for five more minutes, after which the liquid in the right side manometer arm was adjusted to the reference point of 150 mm. After closing the stopcocks readings were taken at five minute intervals by always adjusting the liquid on the closed arm of the manometer to the selected reference point. From these readings both the oxygen uptake and carbon dioxide evolution could be calculated.

All the Warburg flasks and manometers were calibrated in advance. Based on these values, the flask constants were calculated as shown below. The use of a flask constant enables one to calculate from the observed pressure changes the amount in mm^3 or ul at 0°C and 760 mm Hg pressure, of gas utilized or given off during a given time interval. The following general formula was used for

calculation of flask constants (103):

(1) $$k = \frac{V_g \frac{273}{T} + V_f \alpha}{P_o}$$

where: k = flask constant.
V_g = volume of gas phase in flask including connecting tubes known to the reference point.
V_f = volume of fluid in vessel.
T = temperature of bath in absolute degrees.
α = solubility in reaction liquid of gas involved (expressed as ul gas/ml liquid when gas is at a pressure of 760 mm Hg at the temperature T).
P_o = standard pressure (760 mm Hg) expressed as mm of manometer fluid, = 12.690

Because the retention of carbon dioxide by buffer at pH 5.8 is negligible, one-sidearm vessels could be used. A correction for the retention was made by calculating the flask constants using so-called effective value of α, α'. The values of α'/α as reported by Umbreit et al. (103) were employed.

The amounts of oxygen uptake and carbon dioxide evolution were calculated as follows:

(2) $$x_{O_2} = h_{O_2} k_{O_2}$$

(3) $$x_{CO_2} = (h_{O_2} - \frac{x_{O_2}}{k_{O_2}}) k_{CO_2}$$

where: x_{O_2} = amount of oxygen uptake in microliters.
h_{O_2} = change in the manometer fluid level in the system with potassium hydroxide.
k_{O_2} = flask constant for oxygen uptake measurement.
x_{CO_2} = carbon dioxide evolution in microliters.
h_{CO_2} = changes in the manometer fluid in the system without potassium hydroxide.
k_{CO_2} = flask constant for carbon dioxide evolution measurement.

For the convenience of calculations, the readings and results were tabulated according to the following example:

Flask with KOH					Flask without KOH				
R	D	CD	ul O_2	Total O_2	R	D	CD	ul CO_2	Total CO_2
150					150				
150	0	0	0.00	0.00	159	+ 9	+ 9	+11.934	+11.934
150	0	-1	-1.23	-1.23	172	+13	+12	+17.222	+29.156
149	-1	-1	-1.23	-2.46	183	+11	+11	+15.896	+45.052
149	0	0	0.00	-2.46	194	+11	+11	+14.586	+59.638
148	-1	-1	-1.23	-3.69	203	+ 9	+ 9	+13.244	+72.882
147	-1	-1	-1.23	-4.92	213	+10	+10	+14.570	+87.452

where: R = observed readings at five minute intervals.
D = difference between two readings.
CD = corrected difference for thermobarometer readings.

All Warburg determinations were performed in triplicates.

Glutamic Acid Decarboxylase

The standard Warburg techniques as previously described were applied. The enzyme source consisted of 500 mg of ground wheat grains or 100 mg of ground germ. One millliliter of 0.1 molar (14.713 mg/ml) glutamic acid in phosphate buffer of pH 5.8 was pipetted into the sidearm. The pH was adjusted by adding small amounts of solid potassium hydroxide into the stirred solution.

Carboxylase

The enzyme source consisted of 500 mg of ground wheat grains. One milliliter of 0.1 molar (11.005 mg/ml) sodium pyruvate of pH 5.8 was pipetted into the sidearm. The standard Warburg techniques were used.

Effect of Substrate Amount on Glutamic Acid Decarboxylase

The amounts of ground grains (sample 6, second series) employed, were 200,

250, 300, 350, 400, and 500 mg, respectively. One milliliter of 0.1 molar glutamic acid solution of pH 5.8 was used in all determinations. The standard Warburg techniques were used. All determinations were performed in five replicates.

Tetrazolium (TTC) Test

Topographical test. A modified procedure of Lakon (55) was used. The kernels were presoaked overnight in distilled water at +4°C. It was found advisable to use this low temperature during the soaking period to avoid oversoftening of kernels. This enabled making an even lengthwise cut with a razor blade without breaking or crumbling the embryo. It was important to make the cut so that the embryo would be divided symmetrically, and essential parts of the embryo exposed for observation. One hundred seeds were examined in each test. Grains were treated with 1 percent 2,3,5-triphenyl tetrazolium chloride in pH 7.3 phosphate buffer in glass petri dishes. Every dish contained 100 seed-halves. Enough solution was poured in to cover the bisected grains. The samples were allowed to stand for 12 hours in a dark cabinet at room temperature (25°C). Evaluation of staining was done by judging the coloring of embryos. Only kernels with completely stained embryo and scutellum were judged as viable.

Colorimetric test. A modified procedure of Soerger-Domenigg et al. (96) was used. One gram (dry wt.) of ground wheat grains or 250 mg of wheat germ were weighed into test tubes, after which 5 ml of 1 percent TTC-solution were pipetted in the tubes. The stoppered tubes were placed in an oven at 38°C for exactly 1 hour. After the digestion the samples were transferred into an erlenmeyer flask with 25 ml of acetone, and allowed to stand overnight in a dark drawer. The mixtures were then filtered through Whatman No. 5 filter

paper. The absorbency of the extracts was measured with Beckman Model DU spectrophotometer at 520 mu.

Fluorescence

A slightly modified procedure of Cole and Milner (22) was used. Duplicate one gram samples of ground wheat grains or 250 mg samples of wheat germ, were weighed in 250 ml erlenmeyer flasks, after which 25 ml of 0.2 M hydrochloric acid were pipetted. The flasks were occasionally shaken by hand, and allowed to stand overnight at room temperature (25°C). The mixtures were filtered through Whatman No. 5 filter paper, after which the filtrates were diluted if found necessary and used as such for fluorescence determination.

Measurements were made with Coleman Photoelectric Fluorometer, using B_1-S and PC-1 filters. Apparatus was balanced to zero with 0.2 M hydrochloric acid and standardized to read 60 with 0.1 ppm sodium fluorescein solution.

Fat Acidity

Fat acidity was determined according to the AACC, Cereal Laboratory Methods (14). The wheat samples were ground in the Waring Blendor for two minutes. Ten grams (dry wt.) of material were placed in a paper thimble immediately after grinding and extracted in the Goldfish extractor with 50 ml of Skelly Solve (petroleum ether) for six hours. After removal of the solvent, the extract was dissolved in 25 ml of isopropylalcohol benzene mixture, and titrated with 0.0129 N KOH to distinct pink color. Phenolphthalein was used as an indicator. All determinations were performed in duplicates, and corrections were made for blank.

Fat acidity was reported as mg of KOH required to neutralize the free fatty acids in 100 grams of ground wheat and calculated as follows:

(4) $$\text{Fat acidity} = \text{ml KOH} \times \text{N(KOH)} \times 56.1 \times \frac{100}{10}$$

Statistical Methods

Simple correlation coefficients and regression lines were calculated according to Snedecor (91). The reproducibility of the Warburg measurements was determined by calculating intraclass correlation coefficients of all triplicate runs in a series according to Fisher (31). Standard deviations were calculated by the method of the least squares.

RESULTS

Statistical Evaluation of Warburg Determinations

In order to evaluate the replicability of the Warburg measurements, the intraclass correlation coefficients (r_I) were calculated for 25 determinations with ground kernels and ground germ, respectively, each run in three replications. The following intraclass correlation coefficients were obtained:

Whole kernels (500 mg): $r_I = 0.984^{***}$

Germ ends (100 mg) : $r_I = 0.978^{***}$

Both of these values are highly significant ($P < 0.001$), but they are not significantly different from each other. Hence the replicability of the Warburg measurements is very good in both cases, and there is no marked difference between the two methods of determining the carbon dioxide evolution from glutamate.

Effect of Sample Size on Warburg Measurements

Runs with different sample sizes were performed in order to determine the optimal substrate amount for Warburg measurements. The following amounts of

ground wheat kernels were used: 200, 250, 300, 350, 400, and 500 mg, respectively. All measurements were performed in five replicates, and a different flask-manometer pair was used for each replicate. As shown in Figure 1, the smallest standard deviation was obtained using a sample size of 300 mg. This standard deviation was significantly smaller than those with 200, 250, 350, 400, and 500 mg samples. Hence 300 mg sample should be the optimum for these measurements. Apparently amounts smaller than 300 mg are too low to give reproducible values for the samples tested. When samples bigger than 300 mg were used, an even wetting with 1 ml of glutamate solution was difficult to obtain. The mixing during the measurement with these samples was also ineffective. However, due to earlier experience (16) and satisfactorily high reproducibility, 500 mg samples were employed throughout these experiments.

Viability Determinations in Series 1
("Sick" Wheat)

The results obtained with "sick" wheat samples are summarized in Tables 2 and 3.

"Sick" wheat. The amount of "sick" wheat varied from 19 to 100 percent, with the bulk of the samples in the range from 31 to 75 percent. All these values are of higher magnitude than those originally given by the grain inspector, Grain Division, Agricultural Marketing Service, Kansas City. These differencies might be at least in part due to additional deterioration during storage in cold room, but most likely they reflect variations in estimation of the germ damage. Table 3 shows the correlation coefficients between the different measurements. A significant highly negative correlation was observed between the amount of "sick" wheat and germination percentage ($r = -0.969^{***}$, Fig. 2) or topographical tetrazolium test ($r = -0.973^{***}$). Correlation

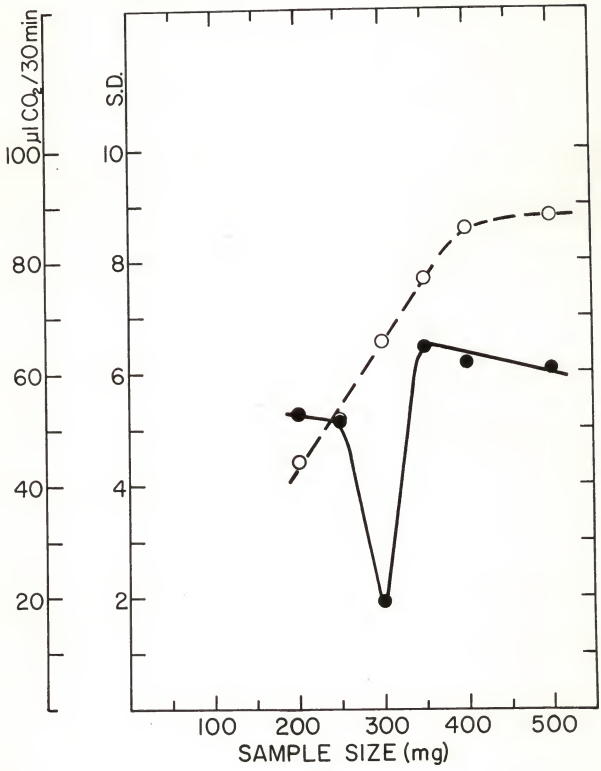

Fig. 1. Effect of substrate amount on the standard deviation of decarboxylase measurements with 500 mg of whole ground kernels. • = standard deviation; o = total CO_2 output.

Table 2. Results from viability tests with "sick" wheat samples (series 1).

Sample No.	Germination	Sick Wheat: Author	Sick Wheat: Original[1]	Fat Acidity	TTC-Test: Colorimetric: Topogr.	TTC-Test: Colorimetric: Germ	TTC-Test: Colorimetric: Kernel	TTC-Test: Fluorescence: Germ	TTC-Test: Fluorescence: Kernel	CO_2-Evolution: Germ	CO_2-Evolution: Kernel
	%	%	%	mg KOH/100g	%	E/250mg	E/1000mg	(250mg)	(1000mg)	ul/100mg	ul/500mg
1	90	19	5	19.455	88	0.129	0.378	32	30	69.381	82.699
2	86	24	7	20.405	84	0.187	0.385	37	32	66.769	80.885
3	82	25	10	25.739	86	0.193	0.430	32	30	77.010	93.398
4	92	19	13	20.089	89	0.141	0.420	25	30	78.522	95.793
5	65	31	15	27.495	65	0.071	0.283	37	20	36.168	44.266
6	66	36	16	26.995	62	0.078	0.318	60	32	46.303	70.747
7	66	37	18	26.053	71	0.075	0.250	40	35	40.515	43.131
8	78	32	20	25.615	73	0.084	0.245	27	27	46.223	65.341
9	48	38	23	38.417	51	0.034	0.178	35	40	24.029	34.076
10	47	54	25	40.992	49	0.035	0.205	37	37	29.241	38.187
11	57	50	28	31.074	60	0.070	0.273	42	27	26.770	39.018
12	39	67	30	42.058	41	0.044	0.160	37	35	12.860	18.902
13	58	43	33	31.893	57	0.068	0.270	40	40	46.417	58.255
14	41	43	35	36.600	45	0.045	0.138	42	35	17.139	32.295
15	51	53	38	28.880	54	0.088	0.350	75	35	35.951	47.969
16	75	34	40	29.380	75	0.055	0.172	32	37	55.468	85.188
17	36	57	45	44.257	37	0.033	0.109	47	42	21.974	31.711
18	44	64	48	31.702	42	0.050	0.150	105	40	26.329	42.367
19	58	42	50	31.893	53	0.045	0.200	40	32	30.483	42.807
20	17	75	55	42.686	22	0.029	0.093	55	45	12.818	28.964
21	33	59	60	38.922	47	0.054	0.140	52	37	31.673	45.809
22	2	94	75	41.621	3	0.027	0.050	157	45	2.798	11.014
23	0	100	98	41.744	1	0.031	0.030	250	110	2.215	5.013
24	0	100	99	45.200	0	0.029	0.030	250	83	0.686	4.550
25	0	99	100	43.191	0	0.027	0.024	250	90	2.032	4.396

[1] Grain Division, Agricultural Marketing Service, Kansas City, Missouri.

Table 3. Simple correlation coefficients between various viability determinations with "sick" wheat samples (series 1).[1]

	B	C_1	C_2	D_1	D_2	E_1	E_2	E_3	F
A	-0.969	-0.882	-0.878	0.858	0.804	-0.973	-0.690	-0.859	0.837
B		0.933	0.921	-0.791	-0.758	0.990	0.766	0.902	-0.906
C_2		0.981			-0.637	0.912		0.873	-0.864
D_2				0.920					0.590
E_1							0.766	0.902	

A = "sick" wheat percentage
B = Germination percentage
C_1 = ul CO_2 by 100 mg of germ ends from 1 ml of 0.1 M glutamate in 30 min.
C_2 = ul CO_2 by 500 mg of whole kernels from 1 ml of 0.1 M glutamate in 30 min.
D_1 = Fluorescence by 250 mg of germ ends
D_2 = Fluorescence by 1000 mg of whole kernels
E_1 = Topographical TTC-test
E_2 = Colorimetric TTC-test with 250 mg of germ ends
E_3 = Colorimetric TTC-test with 1000 mg of whole kernels
F = Fat acidity

[1] All coefficients are significant at 0.1 percent level ($P < 0.001$)

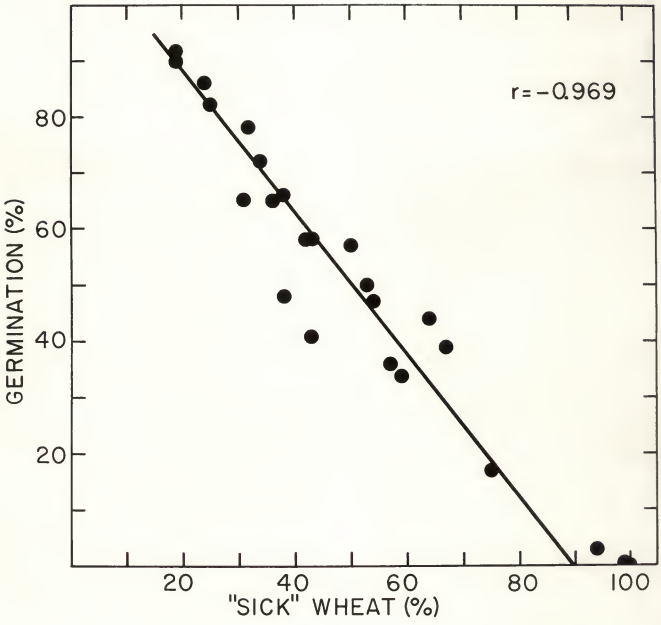

Fig. 2. Relationship between viability and percent of "sick" wheat for 25 samples of commercial hard red winter wheat (series 1).

coefficients between "sick" wheat and Warburg determinations (Figs. 3 and 4) were also higher than those between the amount of "sick" wheat and fluorescence or fat acidity, respectively.

Fat acidity. Fat acidity is reported as mg of potassium hydroxide required to neutralize free fatty acids from 100 grams of grain (dry basis). The fat acidity varied from 19.455 to 45.200. As can be seen from Table 3 and Figure 5, fat acidity has a highly negative correlation with germination percentage ($r = -0.906^{***}$) and with Warburg measurements ($r = -0.864^{***}$), and a relatively high positive correlation with the amount of "sick" wheat ($r = 0.837^{***}$). The correlation between fat acidity and fluorescence is much poorer ($r = 0.590^{***}$).

TTC-Tests. The results from the three different types of TTC-tests are given in Tables 2 and 3, and Figures 6 and 7. The correlations between topographical TTC-test and germ-damage ($r = -0.973^{***}$) or germination percentage ($r = 0.990^{***}$) are very significant. It is important to notice that a high positive correlation exists between topographical TTC-test and Warburg test with whole kernels ($r = 0.912^{***}$). Colorimetric TTC-tests with ground whole kernels and ground germ ends, respectively, show somewhat poorer, but still significant correlations with "sick" wheat and germination percentage than does topographical TTC-test.

Fluorescence. There is a high positive correlation ($r = 0.920^{***}$) between the fluorescence measurements with ground kernels and ground germ ends. Hence only ground whole kernels were used during later experiments. In general, the correlation between fluorescence and "sick" wheat is somewhat higher than that between fluorescence and germination percentage, as shown by Table 3, and Figures 8 and 9. The negative correlation between fluorescence and Warburg determinations is rather low ($r = -0.637^{***}$).

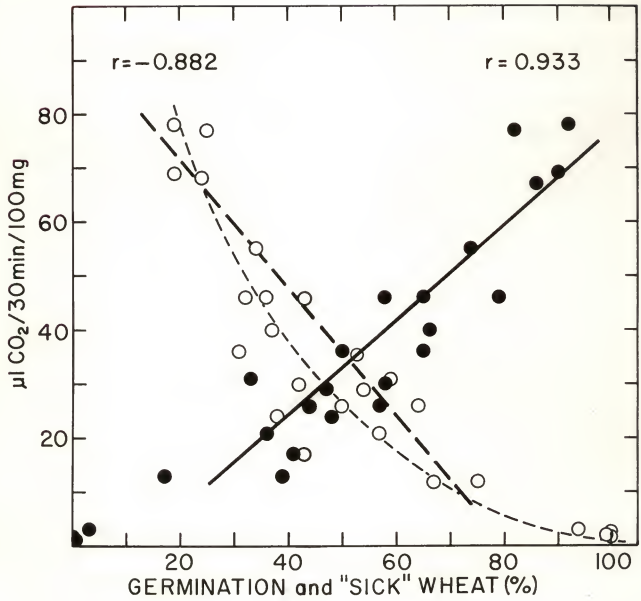

Fig. 3. Correlations between decarboxylation of glutamate of 100 mg of germ ends, and germination percentage, or percent of "sick" wheat (series 1). • = germination; o = "sick" wheat.

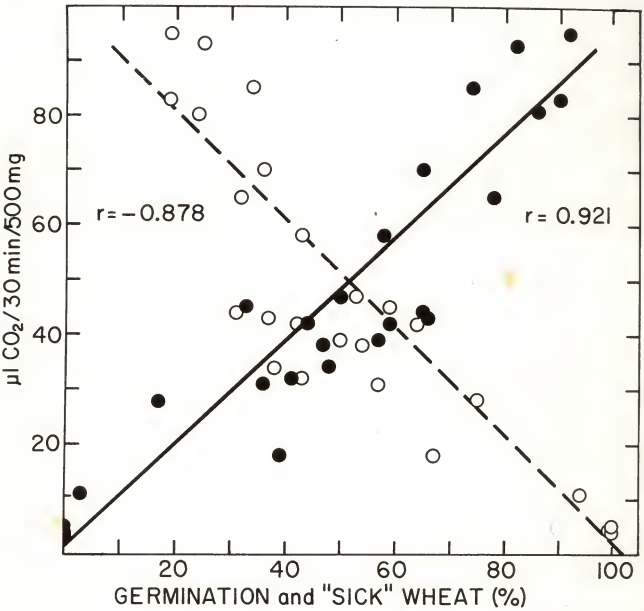

Fig. 4. Correlations between decarboxylation of glutamate of 500 mg of whole ground kernels, and germination percentage, or percent of "sick" wheat (series 1). • = germination; o = "sick" wheat.

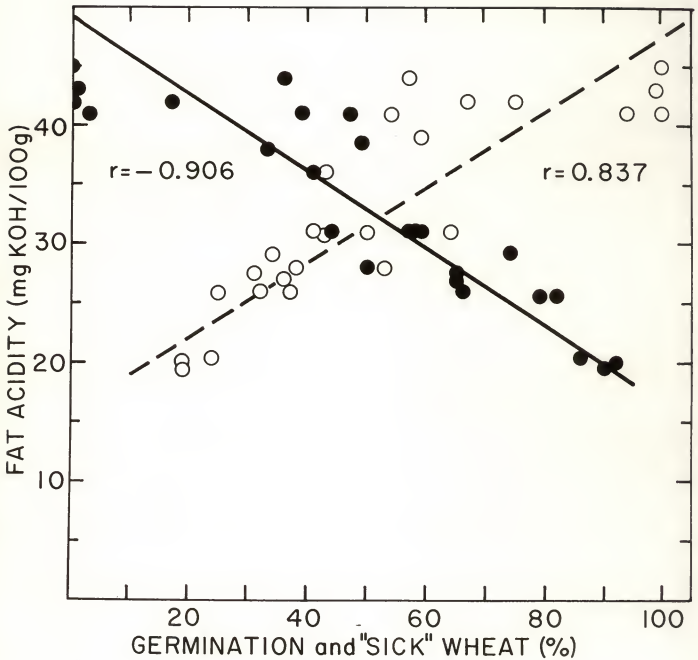

Fig. 5. Correlations between fat acidity and germination percentage, or percent of "sick" wheat (series 1). ●=germination; ○ = "sick" wheat.

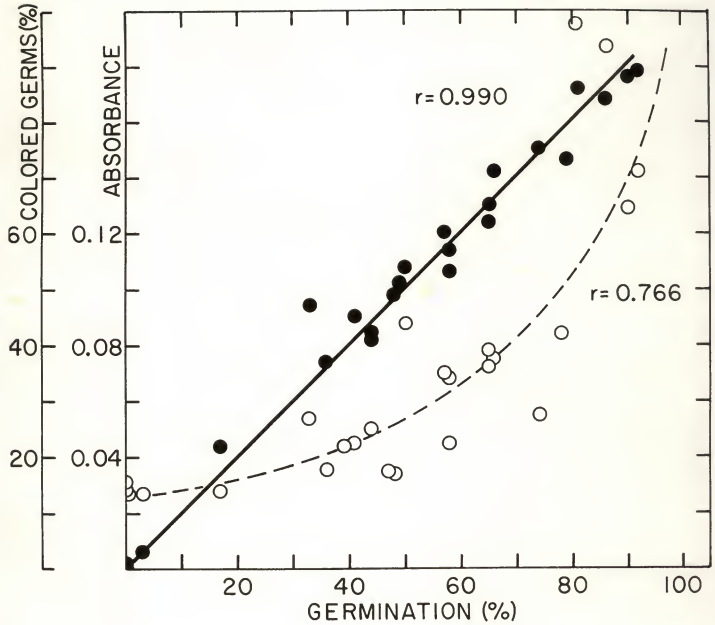

Fig. 6. Correlations between germination percentage and colorimetric TTC-test with 250 mg of ground germ ends, or topographical TTC-test, respectively (series 1). • = % colored germs; o = absorbance.

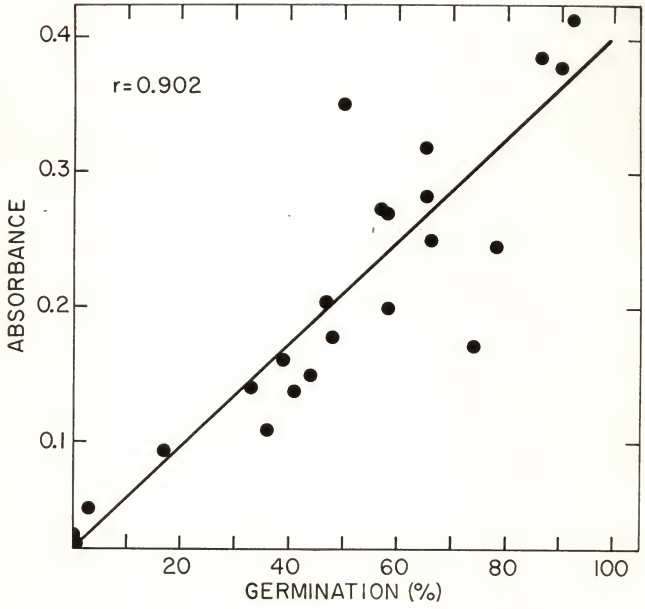

Fig. 7. Correlation between germination percentage and colorimetric TTC-test with 1 gram of ground whole kernels (series 1).

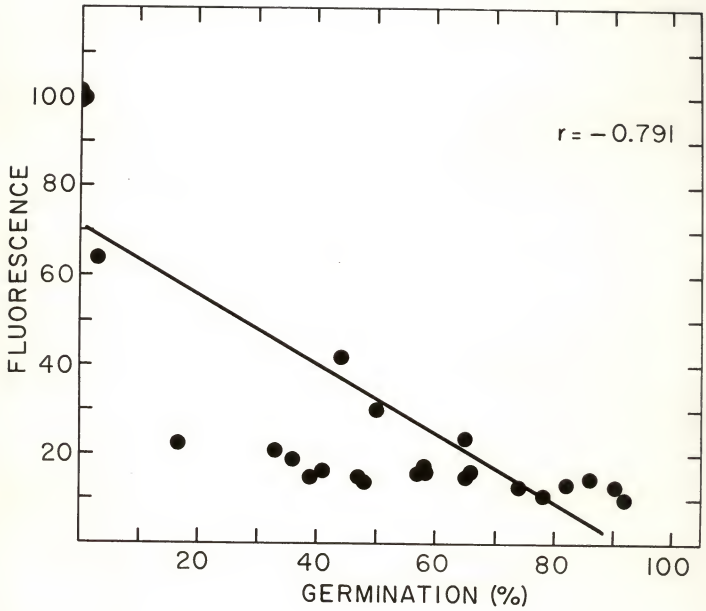

Fig. 8. Correlation between germination percentage and fluorescence of 1 gram of ground whole kernels (series 1).

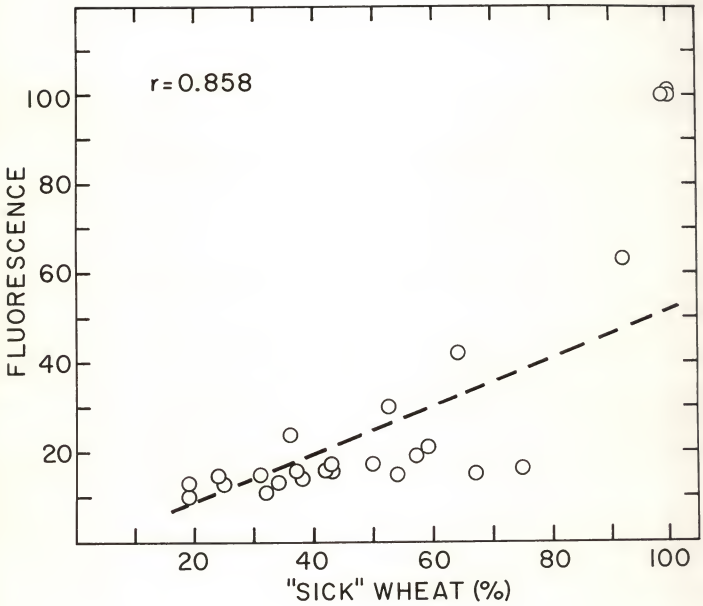

Fig. 9. Correlation between percent "sick" wheat and fluorescence of 1 gram og ground whole kernels (series 1).

Warburg determinations. The carbon dioxide values reported are averages of three replicate determinations. Carbon dioxide evolution from 1 ml of 0.1 M glutamate varies from 0.686 to 78.522 ul with 100 mg of germ, and from 4.396 to 95.793 ul with 500 mg of ground kernels. Correlation between both of these two carbon dioxide measurements is highly positive ($r = 0.981^{***}$); hence only whole ground kernels were used during later experiments. As shown by Figure 4, correlation between decarboxylation of glutamate by whole kernels and germination percentage is highly positive ($r = 0.921^{***}$). Somewhat lower, though significant, negative correlation was found between decarboxylation of glutamate and the amount of "sick" wheat ($r = -0.878^{***}$).

It has been claimed that pyruvate is less affected than glutamate by varietal differences (15). In order to investigate this problem, glutamate was substituted with pyruvate in Warburg determinations. These runs were also performed using 500 mg of ground whole kernels. Five samples from each of the two series were picked out randomly. As indicated by Tables 4 and 5, generally somewhat lower values were obtained for carbon dioxide evolution from pyruvate than from glutamate, but results were otherwise rather parallel to those obtained by using glutamate. Correlation coefficients were calculated separately for the five samples from series 1 and the five samples from series 2. Correlation coefficients obtained are presented in Table 6. Similar results were obtained with pyruvate and glutamate when "sick" wheat samples (series 1) were employed. The correlation coefficients obtained with series 2 are not significant, though correlation of germination percentage with decarboxylation of pyruvate ($r = 0.494^{ns}$) is markedly higher than that with glutamate ($r = -0.088^{ns}$). As can be seen from Table 10, correlation between germination percentage and decarboxylation of glutamate in series 2 is relatively high ($r = 0.744^{***}$). Therefore it is quite clear that the number of samples used

Table 4. The relationship of carboxylase activity to various viability determinations in wheats of series 1.

Sample No.	Germination percent	Sick Wheat percent	Fat Acidity mg KOH/100g	Topographical TTC-test percent	Fluorescence Whole Kernel (1000mg)	CO_2-Evolution Whole Kernel (500mg) Glutamate	CO_2-Evolution Whole Kernel (500mg) Pyruvate
1	90	19	19.455	88	30	82.699	65.804
8	78	32	25.615	73	27	65.341	56.274
15	51	53	28.880	54	35	47.969	49.575
18	44	64	31.702	42	40	42.367	47.461
20	17	75	42.686	22	45	28.964	25.976

Table 5. The relationship of carboxylase activity to various viability determinations in wheats of series 2.

Sample No.	Germination percent	Sick Wheat percent	Fat Acidity mgKOH/100g	Topographical TTC-test percent	Fluorescence Whole Kernel (1000mg)	CO_2-Evolution Whole Kernel (500 mg) Glutamate	CO_2-Evolution Whole Kernel (500 mg) Pyruvate
7	83	2	13.22	92	40	103.705	88.679
11	77	13	11.22	94	50	95.216	80.944
15	80	8	13.39	95	27	64.726	75.881
19	96	6	12.11	96	17	71.428	86.720
24	97	4	9.77	95	11	127.899	97.042

Table 6. Comparison of the decarboxylation of pyruvate and glutamate. Correlation coefficients.

	Series 1		Series 2	
	Pyruvate	Glutamate	Pyruvate	Glutamate
"Sick" wheat, %	-0.940^{**}	-0.759^{*}		
Germination, %	0.973^{**}	0.985^{***}	0.494^{ns}	-0.088^{ns}

in pyruvate decarboxylation studies was far too small, and that completely different results might have been expected if another five samples, or especially if more samples would have been studied.

Viability Determinations in Series 2

The results obtained are shown in Tables 7 and 8. The differences in viability among these samples were unfortunately relatively small. With the exception of sample 20 with no germination, germination percentage varied from 78 to 96. Hence no great differences were encountered in fat acidity and fluorescence, either. There was a little more variation in carbon dioxide evolution from glutamate, which may be due to varietal differences, or damages which are not detectable by tests performed in this study. Despite this fact, the correlation coefficients shown in Table 8 are all significant at 0.1 percent level. Fat acidity and fluorescence showed a very high negative correlation with germination percentage ($r = -0.920^{***}$, and $r = -0.957^{***}$, respectively). In this series, correlation between decarboxylation of glutamate and germination percentage was rather low ($r = 0.744^{***}$).

Viability Determinations of Frost Damaged Wheats

The results are presented in Tables 9 and 10. One can see that germination percentage decreases with an increasing relative amount of frost damage. At the same time "sick" wheat percentage, fat acidity and fluorescence increase considerably. The positive correlation between the topographical TTC-test and the germination test is high ($r = 0.996^{***}$). The negative correlation obtained between decarboxylation of glutamate and germination percentage is not significant.

Table 7. Results from viability tests with wheats of series 2.

Sample No.	Germination percent	Sick Wheat percent	Fat Acidity mg KOH/100g	Topographical TTC-test percent	Fluorescence Kernels (1000mg)	CO_2-Evolution Kernels ul/500mg
5	92	3	11.94	96	28	92.541
6	92	4	11.94	93	26	87.633
7	83	2	13.22	92	40	103.705
8	83	1	12.77	95	42	118.236
9	88	23	12.66	96	19	95.239
10	84	22	9.05	90	21	102.275
11	77	13	11.22	94	50	95.216
12	80	12	10.49	93	36	105.737
13	89	11	17.73	92	47	114.774
14	90	15	14.90	92	36	119.599
15	80	8	13.39	95	27	64.726
16	78	20	11.94	96	29	67.586
17	92	12	11.11	96	20	92.336
18	94	12	11.94	95	16	101.659
19	96	6	12.11	96	17	71.428
20	0	60	45.23	0	188	7.478
21	81	9	12.13	94	12	65.400
22	88	7	12.66	95	17	116.971
23	90	11	11.94	97	11	105.581
24	97	4	9.77	95	11	127.899

Table 8. Simple correlation coefficients between various viability determinations with wheats of series 2.[1]

	B	C	D	E	F
A	-0.867	-0.682	0.830	-0.876	0.825
B		0.744	-0.957	0.971	-0.920
C			-0.656	0.797	-0.696

A = Percent damaged kernels
B = Percent germinating kernels
C = ul CO_2 evolution from 500 mg of whole kernels in 30 min.
D = Fluorescence of 1000 mg of whole kernels
E = TTC-test, topographical method; percent stained kernels
F = Fat acidity

[1] All coefficients are significant at 0.1% level ($P < 0.001$)

Table 9. Results from viability tests with frost damaged samples.

Sample No.[1]	Germination percent	Sick Wheat percent	Fat Acidity mg KOH/100g	Topographical TTC-test percent	Fluorescence: Kernel (1000mg)	CO_2-Evolution Kernel ul/500mg
1	96	9	14.84	95	40	93.737
2	88	15	15.56	92	39	106.048
3	85	22	19.18	90	56	104.731
4	74	32	21.35	86	86	103.495

[1] 1 = light frost damage
2 = moderate frost damage
3 = bad frost damage
4 = very bad frost damage

Table 10. Simple correlation coefficients between various viability determinations with frost damaged wheats.

	A	C	D	E	F
B	-0.982^{**}	-0.608^{ns}	-0.926^{*}	0.996^{***}	-0.933^{*}

A = Percent damaged kernels
B = Percent germinating kernels
C = ul CO_2 evolution from 500 mg of whole kernels in 30 min.
D = Fluorescence of 1000 mg of whole kernels
E = Topographical TTC-test, (percent stained kernels)
F = Fat acidity

DISCUSSION

In the present study, the germination test was used as a basis in determining the viability of the wheat samples. Though several new methods have been introduced, the germination test still remains the most widely used and accepted viability test throughout the world. In Western Europe this test is now standardized in regard to temperature, time, and other conditions involved. Hence it is possible to compare results from different seed testing stations, as well as from country to country.

Series 1 covered reasonably well a germination range from 0 to 100 percent, with a few exceptions. Unfortunately this was not so with series 2, in which all germination percentages except sample 20 fell in the area from 77 to 97 percent. Though this was not desirable in the present study, it can be well understood since the majority of samples in this series were of 1959 crop. The germination of frost damaged samples varied from 74 to 96 percent. The results obtained were checked by shipping 200 grams of each sample to the Seed Laboratory, Kansas State Board of Agriculture, Topeka, where official germination tests were performed. Germination percentages thus obtained were in very close agreement with the results of the author.

As can be seen from Table 2, the "sick" wheat percentages obtained by the author as compared to those originally reported by the Grain Division, Agricultural Marketing Service, USDA, Kansas City, differed somewhat from each other. The differences observed might be partially due to the prolonged storage in the cold room (12, 21, 22), and in part to differences in judging the amount of germ-damage. The values obtained in this study were used in statistical calculations, because it was felt that they best represented the extent of damage at the time the experiments were performed. Generally, rather high correlations were found between "sick" wheat, germination, TTC-tests, fat acidity,

fluorescence, and decarboxylation of glutamate, as indicated by Tables 3, 8, and 10. Sorger-Domenigg, et al. (96) have also reported a significant, though a smaller correlation between "sick" wheat, germination, fat acidity, fluorescence and formazan value (colorimetric TTC-test). These values are shown in Table 11. It is particularly interesting that they obtained a relatively low correlation ($r = -0.491^{***}$) between "sick" wheat and germination. This is contrary to results from the present study, where very high correlation was found ($r = -0.969^{***}$ in series 1, and $r = -0.867^{***}$ in series 2). Cole and Milner (22), who studied the relation of fluorescence to germ-damage, also obtained a high correlation ($r = -0.828^{***}$; calcd. from their values).

It has been known for some time that germ-damage in wheat is followed by high fat acidity (5, 6, 7, 8, 38, 44, 69, 70, 76, 95, 96, 107). The fat acidity values in series 1 varied from 19.46 to 45.20, and in series 2 from 9.05 to 45.23. Sorger-Domenigg, et al. (96) reported a somewhat larger distribution with 68 samples of hard red spring and winter wheats, the values varying from 13 to 67. Baker, Neustadt and Zeleny (6) found for 500 commercial wheat samples fat acidity values from 11 to 66 in case of "sick" wheat, and values from 7 to 45 in case of sprout damaged wheat. In general, the values obtained in this study are in good agreement with earlier results. The correlation between fat acidity and germination percentage was very high ($r = -0.906^{***}$ in series 1, and $r = -0.920^{***}$ in series 2). These values agree well with that of Sorger-Domenigg, et al. (96) ($r = -0.915^{***}$). The correlation found in the present work between fat acidity and "sick" wheat percentage was only slightly lower than that involving viability, in contrast to Sorger-Domenigg, et al. who found a rather low correlation ($r = 0.457^{***}$). On the other hand the correlation coefficients $+0.81^{***}$ (5) and $+0.847^{***}$ (6) obtained by Baker and co-workers between fat acidity and "sick" wheat percentage are virtually the same as found

Table 11. Simple correlation coefficients between various properties of sixty-eight commercial wheat samples (Sorger-Domenigg, et al. (96)).[1]

	Fat Acidity	Fluorescence Value	Formazan Value	Germ Damage
Viability	-0.92	-0.66	+0.60	-0.49
Fat acidity		+0.67	-0.56	+0.46
Fluorescence			-0.44	+0.47
Formazan value				-0.69

[1] Value of r at 1 percent point = 0.30

in the present study ($r = 0.837^{***}$ in series 1, and $r = 0.825^{***}$ in series 2). Baker, et al. (6) found a very low correlation between fat acidity and heat damage ($r = 0.651^{*}$), or sprout damage ($r = 0.551^{**}$). These results suggest that increased fat acidity is an indication of lowered viability, though the increase in free fatty acids does not have to be primarily associated with any of the above mentioned deteriorative processes.

Zeleny, et al. (107) distinguish between three types of acidic compounds in cereal grains, namely fatty acids, acid phosphates, and amino acids. He also related these to different degree of deterioration. According to him, acid phosphates and amino acids increase significantly only in wheat samples which have undergone a considerable degree of deterioration, whereas a highly significant increase in fat acidity may appear at a very early stage of deterioration. Glass and Geddes (37) reported recently that deteriorating wheat exhibits an increase in inorganic phosphorus. They suggest that the increase, which is accelerated by moisture and temperature, apparently is due to the action of phytase upon phytic acid. Though they found a greater increase in fat acidity than inorganic phosphorus, the latter increased more rapidly at the early stages of deterioration.

Milner and co-workers (22, 66) have shown that extracts of "sick" wheat embryos exhibit an increase in fluorescence, which precedes the respiratory increase indicative of mold growth. Fluorescence of sound wheat samples of a widely different source, variety and class, was found low and virtually uniform (22). Hence Cole and Milner concluded that increasing fluorescence depends on the condition of the germ, and they suggested the use of a fluorescence value as an indicator of the amount and extent of germ-damage.

In the present work the fluorescence value was determined both with whole ground kernels and with ground germ ends. As shown by Table 3, correlation

between the two measurements is very high ($r = 0.920^{***}$). With a few exceptions there was little change in fluorescence in samples of 100 to 20 percent of germination (Fig. 8) and of 20 to 50 percent of "sick" wheat (Fig. 9). The correlation between fluorescence and germination percentage in all of the 25 samples of series 1 was -0.758^{***} (ground whole kernels). This value is in good agreement with results obtained by Cole and Milner (22) ($r = -0.775^{***}$), and Sorger-Domenigg, et al. (96) ($r = -0.663^{***}$). However, when a correlation coefficient was calculated neglecting the extreme samples 22, 23, 24, and 25 of very high amount of "sick" wheat, poor correlation was obtained ($r = -0.479^{***}$). Neglecting also samples 15 and 18, a correlation -0.663^{***} was found. Similar results were obtained with samples of series 2 (Table 12). The overall correlation between fluorescence and germination percentage was very high ($r = -0.957^{***}$), but after neglecting sample 20 (0 percent germination; 60 percent "sick" wheat) very poor correlation ($r = -0.412^{**}$) was obtained. This suggests that correlation between fluorescence and viability, or "sick" wheat actually is curvlinear. At the early stages of deterioration this is nearly linear. When deterioration advances distribution of fluorescence values obtained with wheat samples of the same viability progressively increases. In particular unexpectedly high values of fluorescence were encountered in samples of little or no viability, and over 60 percent of germ-damaged wheat. This agrees well with the results of Linko, et al. (60) who concluded that primary browning products in "sick" wheat development are not fluorescent; viability may be lost before any browning of germ can be noticed, and visually detectable browning precedes any marked increase in fluorescence. After viability is lost, nonenzymatic browning advances leading to highly fluorescent compounds.

Cheng (15) reported recently a negative correlation between fluorescence and glutamic acid decarboxylase activity. It was also suggested by Cheng,

Table 12. Simple correlations in series No. 2 with sample 20 excluded.

Correlation	Correlation Coefficients	
	All Samples	Without Sample 20
"Sick" wheat and germination	-0.867^{***}	-0.195^{ns}
Warburg test and germination	0.744^{***}	0.185^{ns}
TTC-test and germination	0.971^{***}	0.849^{***}
Fluorescence and germination	-0.957^{***}	-0.412^{**}

et al. (17) that the marked immediate carbon dioxide evolution from wetted wheat germ is due to enzymatic decarboxylation of free glutamic acid. Though glutamic acid decarboxylase is activated by the addition of water, the activity decreases markedly during storage of moist wheat (15, 79). These phenomena are followed by a decrease in free glutamic acid and an increase in free gamma-aminobutyric acid (62). Kiraly and Farkas (48) found that glutamic acid decarboxylase activity was considerably lowered in wheat infected by stem rust (Puccinia graminis).

As can be seen from Tables 2 and 7 there was a relatively large variation in carbon dioxide values among different samples. Though the simple linear correlations, as indicated by Table 2, and Figures 3 and 4 for series 1, between Warburg tests and viability ($r = 0.921^{***}$ for whole kernels; $r = 0.933^{***}$ for germ ends), or "sick" wheat ($r = -0.878^{***}$ for whole kernels; $r = -0.882^{***}$ for germ ends) are high and significant, it appears likely that correlation in the case of "sick" wheat actually may be closer to curvlinear. Enzyme activity decreases most rapidly at the early stages of deterioration, when only a few grains have totally lost their viability. In series 2 (Table 10) correlations between decarboxylation of glutamate and viability ($r = 0.744^{***}$), or "sick" wheat ($r = -0.682^{***}$), respectively, were somewhat poorer. If again sample 20 was neglected in statistical calculations, resulting correlations were insignificant (Table 12). Wheats in this series were obtained from widely different sources, differing as to variety and growth conditions. All were from the 1959 crop. It has been shown previously (15, 16) that significant differences in the activity of glutamic acid decarboxylase occur in different varieties of wheat, and in wheats from different locations. It is therefore most likely that the varietal differences in "new" wheats of high viability are at least of the same order of magnitude as differences due to beginning deteriorative

processes. Though this enzyme reaction seems to have little value in examining "new" wheat samples, it, either alone or together with germination test and determination of the free amino acids, may give an excellent picture of the storage background of wheat.

A preliminary study (15) had shown that there were virtually no varietal differences in regard to carboxylase activity in wheat grains, and that the two reactions were catalyzed by different enzyme systems. As indicated by Table 6 in series No. 1, the correlation coefficients obtained from Warburg tests with pyruvate as substrate were significant and of the same order of magnitude as was found with glutamate. The five samples examined for carboxylase activity in series 2 were chosen so that there was no correlation between decarboxylation of glutamate and germination percentage. The correlation involving pyruvate was numerically greater ($r = 0.494^{ns}$), but insignificant. This may have been entirely due to the small number of samples investigated.

Although the topographical tetrazolium test particularly has been widely accepted as a means to detect viability of seeds, the results also from TTC-tests have been reported to differ with the variety of wheat, location of growth, and the degree of mold infestation (96). Lakon (55), who introduced the TTC-test, found a good agreement between it and germination percentage. Indeed, he even preferred the TTC-test over the conventional germination test because of the short time involved in the former. Several other persons have obtained good results with this method (24, 25, 46, 82, 88). In the present study the topographical TTC-test generally gave the best correlation with both germination percentage and the amount of "sick" wheat (Tables 3 and 8). Even if sample 20 in series 2 was neglected (Table 12) a high correlation with viability was obtained ($r = 0.849^{***}$). The correlations between colorimetric TTC-tests and germination percentage were somewhat lower, though highly significant.

This is in agreement with the relatively low correlation obtained by Sorger-Domenigg, et al. (96) ($r = 0.602^{***}$). Figures 6 and 7 indicate that the correlations between germination percentage and colorimetric TTC-tests may be curvlinear, particularly in the case of germ ends. At very low viability and advanced deterioration, reducing compounds formed may possibly contribute to formazan synthesis.

Another question also arises in connection with the TTC-tests, namely, the accuracy of the method in samples of very high or low germination. The present work does not bring much light to the question. However, Isely (46) has reported surprisingly accurate results with seeds of above 90 percent germination. Results were not so successful for weaker lots. Damage done to other parts of the seed than the embryo will not be detected by the TTC-test, though it may cause the loss of viability. Also an injury resulting from chemical treatment may not be detected. Godsell (39), and Bennett, et al. (9) observed that frost damaged corn, though potentially dead, stained red if it was tested shortly after freezing. However, after drying the corn to a moisture level of 12 percent or less prior to testing a good correlation with germination percentage was generally obtained.

As shown by Table 9 the few frost damaged samples examined exhibited a highly significant correlation between topographical TTC-test and germination percentage ($r = 0.996^{***}$). The correlations between germination percentage and the amount of "sick" wheat, fluorescence, or fat acidity, respectively were significant. This is contrary to the observations of Baker, et al. (6) who found low fat acidity values in all 48 samples studied regardless of the degree and amount of frost damage. They concluded that freezing of wheat does not cause hydrolytic deterioration of fats. However, it seems quite likely that on prolonged storage, deteriorative processes may be even faster in grains

initially affected by frost.

SUGGESTIONS FOR FUTURE RESEARCH

The present study has been mainly concerned with methods and tests for determining viability and storage condition of wheat grain. The results obtained indicate that many of the tests considered seem to be useful and reliable, at least for the samples investigated.

The topographical TTC-test turned out to be a good indicator for viability in this study. However, much still remains unexplored about this test. Do the different wheat varieties and classes react differently to the tetrazolium reagent? What influence has a high relative humidity during harvest, storage conditions, and time of storage on the test? These problems should be studied by performing the TTC-test under carefully controlled conditions on a large series of wheats of different varieties and classes with different artificially induced damages. These results should then be correlated with glutamic and pyruvic acid decarboxylase activities.

While a negative tetrazolium test indicates a deficiency in a seed's reducing systems, it does not identify the site or sites of the breakdown in the respiratory pathway. The enzyme proteins themselves, coenzymes, metal-ions, etc., may be involved. There is also evidence that seeds may have normal tetrazolium reactions or even normal respiratory rates at the early stages of germination and still not grow. The diagnosis of these deficiences, and development of tests to detect or possibly to avoid them, should also be subjected to future research.

The glutamic acid decarboxylase activity showed a high positive correlation with viability, but little is known of how many of the variations in enzyme activity are due to variety, class, location, type of damage, etc. This study

also showed that further examination on the pyruvic acid decarboxylase activity and its relationship to glutamic acid decarboxylase would be of interest. A simple and reliable method, suitable for routine measurement of the decarboxylase activity in grain, should be developed.

Frost damage is rather common in many of the wheat producing countries, and to have a reliable test which could serve to predict actual damage and storage behavior, would be of considerable value. What happens to the enzymes in wheat during freezing would be another worthwhile problem to study in the future.

SUMMARY

In the present work several viability tests have been performed on 49 wheat samples of different origin and history. Twenty-five of the samples were of old germ-damaged wheat (series 1), and 24 were new wheats from the 1959 crop, including 4 samples of frost damaged kernels.

Germination (on moist sand), "sick" wheat, free fat acidity, fluorescence, reduction of 2,3,5-triphenyltetrazolium chloride (TTC, colorimetric and topographical methods), and decarboxylation of glutamate and pyruvate were studied.

Several TTC, fluorescence, and decarboxylation measurements were performed both on ground whole kernels and on ground germ ends. It was found that in all cases ground whole kernels can be successfully employed.

The replicability of Warburg measurements was evaluated by calculating intraclass correlation coefficients from 25 samples, each run performed in triplicate. Coefficients $r_I = 0.984^{***}$ for 500 mg of whole kernels and $r_I = 0.978^{***}$ for 100 mg of germ ends were obtained.

The smallest standard deviation was obtained when 300 mg samples were used. In series 1 germination percentages varied from 0 to 92, and in series

2 from 77 to 98 with the exception of sample No. 20, which did not germinate at all.

In germ-damaged wheat samples, a significantly high negative correlation was found between the amount of "sick" wheat and germination percentage ($r = -0.969^{***}$) or topographical TTC-test ($r = -0.973^{***}$). Correlation coefficients between "sick" wheat and Warburg determinations ($r = -0.878^{***}$, whole kernels) were also higher than those between the amount of "sick" wheat and fluorescence or fat acidity, respectively. Fat acidity had a highly negative correlation with germination percentage ($r = -0.906^{***}$) and with Warburg measurements ($r = -0.874^{***}$, whole kernels), and a relatively high positive correlation with the amount of "sick" wheat ($r = 0.837^{***}$). The correlation between fat acidity and fluorescence was much poorer ($r = 0.590^{***}$). It was interesting to notice that a high positive correlation existed between the topographical TTC-test and the Warburg test with whole kernels ($r = 0.912^{***}$). Colorimetric TTC-tests showed somewhat poorer, though still significant, correlations with "sick" wheat and germination percentage. Correlation between decarboxylation of glutamate and germination percentage was highly positive ($r = 0.921^{***}$).

With wheats of the 1959 crop, fat acidity and fluorescence showed a very high negative correlation with germination percentage ($r = -0.920^{***}$, and $r = -0.957^{***}$, respectively). In this series, correlation between decarboxylation of glutamate and germination percentage was rather low ($r = 0.774^{***}$). This led to the conclusion that though the Warburg test seemed to have little value in examining "new" wheat samples, it either alone or together with the germination test and the determination of the free amino acids may give an excellent picture of the storage background of wheat.

Germination percentage was found to decrease, and "sick" wheat, fat

acidity, and fluorescence to increase, with an increasing relative amount of frost damage. The positive correlation between the topographical TTC-test and the germination test was high ($r = 0.996^{***}$). No significant correlation between decarboxylation of glutamate and germination percentage was found, possibly due to the small number of samples employed.

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LITERATURE CITED

(1) Abderhalden, E., G. Fromme, and P. Hirsch.
Die Bildung von γ-Aminobuttersäre aus l-Glutaminsäure unter den Einfluss von Mikro-organismen. Hoppe-Seyler's Z. Physiol. Chem. 85: 131-135, 1913.

(2) Auhagen, E.
Co-Carboxylase, ein neues Co-Enzyme der alkoholischen Gärung. Z. Physiol. Chem. 204: 149-167, 1932.

(3) Auhagen, E.
Über Co-Carboxylase. Z. Physiol. Chem. 209: 20-26, 1932.

(4) Bailey, C. H., and A. M. Curjar.
Respiration of stored wheat. Jour. Agr. Res. 12: 685-710, 1918.

(5) Baker, Doris, M. H. Neustadt, and L. Zeleny.
Application of the fat acidity test as an index of grain deterioration. Cereal Chem. 34: 226-233, 1957.

(6) Baker, Doris, M. H. Neustadt, and L. Zeleny.
Relationships between fat acidity values and types of damage in grain. Cereal Chem. 36: 307-311, 1959.

(7) Barton, L. V.
Effect of moisture fluctuations on the viability of seeds in storage. Contrib. Boyce Thompson Inst. 13 (2): 35-46, 1943.

(8) Barton, L. V.
Seed storage and viability. Contrib. Boyce Thompson Inst. 17 (2): 87-103, 1953.

(9) Bennet, Norah, and W. E. Loomis.
Tetrazolium chloride as a test reagent for freezing injury of seed corn. Plant Physiol. 24: 162-174, 1949.

(10) Beevers, H.
An L-glutamic acid decarboxylase from barley. Biochem. Jour. 48: 132-137, 1951.

(11) Camien, M. N., L. E. McClure, A. Lepp, and M. S. Dunn.
The preparation of γ-aminobutyric acid by enzymatic decarboxylation of L-glutamic acid. Arch. Biochem. Biophys. 43: 378-380, 1953.

(12) Carter, E. P., and G. Y. Young.
Effect of moisture content, temperature and length of storage on the development of "sick" wheat in sealed containers. Cereal Chem. 22: 418-428, 1945.

(13) Carter, E. P., and G. Y. Young.
Role of fungi in the heating of moist wheat. U.S. Dept. Agr. Circ. 838, 1950.

(14) Cereal Laboratory Methods, 6th ed., Publ. by American Assoc. of Cereal Chem., St. Paul, Minn., 1957.

(15) Cheng, Yu-Yen.
The kinetics and occurrence of wheat glutamic acid decarboxylase. M.S. Thesis. Kansas State University, Manhattan, Kansas, 1959.

(16) Cheng, Yu-Yen, P. Linko, and M. Milner.
Glutamic acid decarboxylase in wheat grains. Suomen Kemistilehti B31, 333-335, 1958.

(17) Cheng, Yu-Yen, P. Linko, and M. Milner.
On the nature of glutamic acid decarboxylase in wheat. Plant Physiol., 1959, in press.

(18) Christensen, C. M.
Fungi on and in wheat seed. Cereal Chem. 28: 408-415, 1951.

(19) Christensen, C. M.
Grain storage studies. XVIII. Mold invasion of wheat stored for sixteen months at moisture contents below 15 percent. Cereal Chem. 32: 107-116, 1955.

(20) Christensen, C. M.
Grain storage studies. XXI. Viability and moldiness of commercial wheat in relation to the incidence of germ damage. Cereal Chem. 32: 507-518, 1955.

(21) Christensen, C. M., and R. F. Drescher.
Grain Storage Studies. XIV. Changes in moisture content, germination percentage and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem. 31: 206-216, 1954.

(22) Cole, E. W., and M. Milner.
Colorimetric and fluorometric properties of wheat in relation to germ damage. Cereal Chem. 30: 378-391, 1953.

(23) Conway, E. J., and E. Mach Donnel.
Carboxylase and carbonic acid. Nature (London) 156: 752 only, 1945.

(24) Cottrell, H. J.
Tetrazolium salt as seed germination indicator. Nature (London) 159: 748 only, 1947.

(25) Cottrell, H. J.
Tetrazolium salt as a seed germination indicator. Am. Appl. Biol. 35: 123-131, 1948.

(26) Crocker, W., and G. T. Harrington.
Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Jour. Agr. Res. 15: 137-174, 1918.

(27) Davis, W. E.
The use of catalase as a means of determining the viability of seeds. Proc. Assoc. Off. Seed Anal. of North America 18: 33-39, 1926.

(28) Dennan, R. F., D. S. Hoare, and E. Work.
Diaminopimelic acid decarboxylase in pyridoxinedeficient Eschericia Coli. Biochem. Biophys. Acta 16: 442-443, 1958.

(29) Detmer, W.
Vergleichende Physiologie des Keimungsprocesses der Samen. 565 Jena, 1880.

(30) Eidmann, F. E.
Ein neuer Weg der Saatgutprüfung. Forsch. dienst. 3: 448-455, 1937.

(31) Fisher, R. A.
Statistical Methods of Research Workers, 12th Ed., Oliver and Boyd, Edinburgh, 1954.

(32) Flemion, F., and H. Poole.
Seed viability tests with 2,3,5-triphenyl tetrazolium chloride. Contr. Boyce Thomp. Inst. 15: 243-258.

(33) French, R. C.
Formation of embryo starch during germination as an indicator of viability and vigor in heat-damaged barley. Plant Physiol. 34: 500-505, 1959.

(34) Gadd, I.
Biochemical tests for seed germination. Proc. Int. Seed Test. Assoc. 16: 235-253, 1950.

(35) Gadd, I., and A. Kjaer.
The applicability of selenium and indigo carmine methods for the testing of frost-fusarium-damaged grain. Proc. Intern. Seed Test. Assoc. 12: 140-149, 1940.

(36) Gale, B. F.
The bacterial amino acid decarboxylases. Adv. in Enzymol. 6: 1-32, 1948.

(37) Glass, R. L., and W. F. Geddes.
Grain storage studies. XXVII. The inorganic phosphorus content of deteriorating wheat. Cereal Chem. 36: 186-190, 1959.

(38) Golubchuk, M., H. Sorger-Domenigg, L. S. Cuendet, C. M. Christensen, and W. F. Geddes.
Grain storage studies. XIX. Influence of mold infestation and temperature on the deterioration of wheat during storage at approximately 12% moisture. Cereal Chem. 33: 45-52, 1956.

(39) Goodsell, S. F.
Triphenyltetrazolium chloride for viability determination of frozen seed corn. Jour. Am. Soc. Agron. 40: 432-442, 1948).

(40) Greer, E. N., and J. B. Hutchinson.
Dormancy in British-grown wheat. Nature 155: 381-382, 1945.

(41) Guinard, B. M., and E. E. Snell.
Pyridoxal phosphate and metal ions as cofactors for histidine decarboxylase. Jour. Am. Chem. Soc. 76: 4745-4747, 1955.

(42) Horning, E. S., and A. H. K. Petrie.
The enzymatic function of mitochondria in the germination of cereals. Proc. Roy. Soc. (London), Ser B 102: 188-206, 1927.

(43) Hummel, B. C. W., L. S. Cuendet, C. M. Christensen, and W. F. Geddes.
Grain storage studies. XIII. Comparative changes in respiration, viability, and chemical composition of mold-free and mold contaminated wheat upon storage. Cereal Chem. 31: 143-150, 1954.

(44) Hunter, I. R., D. F. Houston, and E. B. Kester.
Development of free fatty acids during storage of brown (husked) rice. Cereal Chem. 28: 232-239, 1951.

(45) Hyde, M. B., and H. B. Galleymare.
The subepidermal fungi of cereal grains. II. The nature, identity and origin of the mycelium in wheat. Ann. Appl. Biol. 38: 348-356, 1951.

(46) Isely, D.
Employment of tetrazolium chloride for determining viability of small grain seeds. Jour. Paper No. J.-2240, Iowa Agricultural Experiment Station, Ames, Iowa. 1953.

(47) Justice, O. L.
Seed germination-a historical resume. Association of Official Seed Analysts of North America. Fifty Years of Seed Testing (1908-1958) 35-40, 1958.

(48) Kiraly, Z., and G. L. Farkas.
Infektions bedingte Änderung der Glutaminsäuredecarboxylase Aktivität beim rostfallenen Weizen. Naturwiss. 44: 353 only, 1956.

(49) Krebs, H. A., and F. J. W. Roughton.
Carbonic anydrase as a tool in studying the mechanism of reactions involving H_2CO_3, CO_2 or HCO_3^-. Biochem. Jour. 43: 550-555, 1948.

(50) Krishnaswamy, P. R., and K. V. Giri.
Glutamic acid decarboxylase in Rhodotorula glutinis. Bioch. Jour. 62: 301-303, 1956.

(51) Kuhn, R., and D. Jerchel.
Über Invertseifen. VIII. Mitteil. Reduction von Tetrazolium salzen durch Bakterien, Hafe und Keimende Samen. Ber. deut. chem. Ges. 74: 949-952, 1941.

(52) Kulakarni, L., and K. Schonie.
Glutamic acid decarboxylase in legumes. Nature 178: 925 only, 1956.

(53) Lakon, G.
Das Schwinden der Keimfähigkeit der Samen, insbesondere der Getreidefruchte. Ber. deut. bot. Ges. 57: 191-203, 1940.

(54) Lakon, G.
Die topographische Selen methode, ein neues Verfahren zur Feststellung der Keimfähigkeit der Getreodefrüchte ohne Keimversuch. Proc. Int. Seed Test. Assoc. 12: 1-18, 1940.

(55) Lakon, G.
Topographischer Nachweis der Keimfähigkeit der Getreidefrüchte durch Tetrazoliumsalze. Ber. deut. bot. Ges. 60: 299-305, 1942.

(56) Lakon, G.
Topographischer Nachweis der Keimfähigkeit von Mais durch Tetrazoliumsalze. Ber. deut. bot. Ges. 60: 434-444, 1942.

(57) Lakon, G.
The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol. 24: 389-394, 1949.

(58) Lakon, G.
Weitere Forschungen über das Topographical Tetrazolium Verfahren und die Ermittlung der Triebkraft. Proc. Int. Seed Test. Assoc. 16: 254-266, 1950.

(59) Leggatt, C. W.
A further note on catalase activity as a measure of seed viability. Canadian Jour. of Res. 9: 571-573, 1933.

(60) Linko, P., Yu-Yen Cheng, and M. Milner.
Role of soluble carbohydrates in browning of wheat embryos during storage. Cereal Chem., in press.

(61) Linko, P., and M. Milner.
Enzyme activation in wheat grains in relation to water content. Glutamic acid-alanine transaminase and glutamic acid decarboxylase. Plant Physiol. 34: 392-396, 1959.

(62) Linko, P., and M. Milner.
Free amino and keto acids of wheat grains and embryos in relation to water content and germination. Cereal Chem. 36: 280-294, 1959.

(63) Linko, P., and M. Milner.
Gas exchange induced in dry wheat embryos by wetting. Cereal Chem. 36: 274-279, 1959.

(64) Linko, P., and A. I. Virtanen.
On the biosynthesis of some recently discovered derivatives of glutamic acid in plants. Acta Chem. Scand. 12: 68-78, 1958.

(65) Mar, F.
Amylase activity as an indicator of seed viability. M.S. Thesis, Iowa State College, 1944.

(66) McDonald, C. E., and M. Milner.
The browning reaction in wheat germ in relation to "sick" wheat. Cereal Chem. 31: 279-295, 1954.

(67) Morinaga, T.
Effect of alternating temperatures upon the germination of seeds. Amer. Jour. Bot. 13: 141-158, 1926.

(68) Meister, A.
Biochemistry of the amino acids. Academic Press, Inc., New York, N.Y. 1957.

(69) Milner, M., C. M. Christensen, and W. F. Geddes.
Grain storage studies. V. Chemical and microbiological studies on "sick" wheat. Cereal Chem. 24: 23-38, 1947.

(70) Milner, M., C. M. Christensen, and W. F. Geddes.
Grain storage studies. VI. Wheat respiration in relation to moisture content, mold growth, chemical deterioration, and heating. Cereal Chem. 24: 182-199, 1947.

(71) Najjar, V. A., and J. Fischer.
Studies on glutamic acid decarboxylase from Escherichia coli. Jour. Biol. Chem. 206; 215-219,(1954.

(72) Naylor, A. W., and N. E. Tolbert.
Physiol. Plantarum 9: 220, 1956; in R. P. Smith "Glutamic acid decarboxylase and 4-aminobutyric acid in some biological systems". Ph.D. dissertation, Purdue University, 1956.

(73) Neubauer, O., and K. Z. Fromherz.
Über den Abbau der Aminosäuren bei der Hefegärung. Z. Physiol. Chem. 70: 326-250, 1910-1911.

(74) Neuberg, C., and L. Karczag.
Pyruvic carboxylase. In Chemistry and Methods of Enzymes, Part 5, 365. Ed. by Sumner and Somers. New York Academic Press Inc., 1953.

(75) Okunuki, K.
Bot. Mag. (Tokyo) 51: 270, in C. Hoffman-Ostenhof Enzymologie, Wien, Springer Verlag, 1954.

(76) Papavizas, G. C., and C. M. Christensen.
Grain storage studies. XXVI. Fungus invasion and deterioration of wheats stored at low temperatures and moisture contents of 15-18 percent. Cereal Chem. 35: 27-34, 1958.

(77) Porter, R. H., Mary Durell, and H. J. Romm.
The use of 2,3,5-triphenyl tetrazolium chloride as a measure of seed germinability. Plant Physiol. 22: 149-159, 1937.

(78) Roberts, E., and S. Fraenkel.
γ-aminobutyric acid in brain: its formation from glutamic acid. Jour. Biol. Chem. 187: 55-63, 1950.

(79) Rohrlich, M.
Glutaminsäuredecarboxylase im Getreide. Getreide u. Mehl 7: 89-91, 1957.

(80) Rohrlich, M.
Untersuchungen über die Glutaminsäuredecarboxylaseaktivität. Z. Lebensm.-Unters. u.-Forsch. 105: 189-194, 1957.

(81) Rohrlich, M., and R. Rasmus.
Untersuchungen über die Glutaminsäredecarboxylase im Getreide. I. Papierchromatographischer Nachweis der Bildung von γ-Aminobuttersäure. Z. Lebensm.-Unters. u.-Forsch. 104: 313-316, 1956.

(82) Ritvanen, T.
Vorbereitende Versuche zur Anwendung von Tetrazolium chlorid bei Schnellbestimmung der Keimfähigkeit von Thimothee. Jour. Sci. Agr. Soc. Finland, 25: 153-159, 1953.

(83) Sachs, J.
Abhängigkeit der Keimung von der Temperatur. Jahrb. Wiss. Bot. 2: 338-377, 1860.

(84) Schales, O.
Amino acid decarboxylases. In The Enzymes, Vol. II (2), 216 Ed. by Sommer and Myrbäck. Academic Press Inc., New York. 1951.

(85) Schales, O., V. Mims, and S. S. Schales.
Glutamic acid decarboxylase of higher plants. I. Distribution; preparation of clear solutions; nature of prosthetic group. Arch. Biochem. 10: 455-465, 1946.

(86) Schales, O., and S. S. Schales.
Glutamic acid decarboxylation in higher plants. II. pH-activity curve, reaction kinetics, inhibition by hydroxylamine. Arch. Biochem. 11: 155-166, 1946.

(87) Schales, O., and S. S. Schales.
Decarboxylations and transaminations in extracts of higher plants. Arch. Biochem. Biophys. 69: 373-339, 1957.

(88) Shuel, R. W.
Seed germinability tests with 2,3,5-triphenyl tetrazolium chloride. Sci. Agric. 28: 34-38, 1948.

(89) Smith, F. G.
The mechanism of the tetrazolium reaction in corn embryos. Plant Physiol. 27: 445-456, 1952.

(90) Smith, F. G., and G. O. Thorneberry.
The tetrazolium test and seed viability. Proc. Assoc. Off. Seed Analysts, 41: 105-108, 1951.

(91) Snedecor, G. W.
Statistical Methods of Research Workers, 12th. Ed. Oliver and Boyd, Edinburgh, 1954.

(92) Steward, F. C., and G. S. Bidwell.
Protein metabolism, respiration, and growth. A synthesis of results from the use of C^{14}-labeled substrates and tissue cultures. Nature 178: 734-738, 1956.

(93) Steward, F. C., and J. F. Thompson.
The nitrogenous constituents of plants with special reference to chromatographic methods. Ann. Rev. Plant. Physiol. 1: 233-264, 1950.

(94) Steward, F. C., J. F. Thompson, and C. E. Dent.
γ-Aminobutyric acid: a constituent of the potato tuber? Science 110: 439-440, 1949.

(95) Sorger-Domenigg, H., L. S. Cuendet, C. M. Christensen, and W. F. Geddes.
Grain storage studies. XVII. Effect of mold growth during temporary exposure of wheat to high moisture content upon the development of germ damage, and other indices of deterioration during subsequent storage. Cereal Chem. 32: 270-285, 1955.

(96) Sorger-Domenigg, H., L. S. Cuendet, and W. F. Geddes.
Grain storage studies. XX. Relation between viability, fat acidity, germ damage, fluorescence value, and formazan value of commercial wheat samples. Cereal Chem. 32, 499-506, 1955.

(97) Suzuki, Y., and N. Takakuwa.
Decarboxylation of L-glutamic and in Scopolia japonica. Naturwiss. 44: 353-354, 1957.

(98)n Thompson, J. F., J. K. Pollard, and F. C. Steward.
Investigations of nitrogen compounds and nitrogen metabolism in plants. III. γ-Aminobutyric acid in plants, with special reference to the potato tuber and a new procedure for isolating amino acids other than α-amino acids. Plant Physiol. 28: 401-414, 1953.

(99) Thorneberry, G. O.
Some aspects of the tetrazolium reaction in seeds and the enzymes involved. M.S. Thesis, Iowa State College, Ames, Iowa. 1952.

(100) Tuite, J. F., and C. M. Christensen.
Grain storage studies. XVI. Influence of storage conditions upon the fungus flora of barley seed. Cereal Chem. 32: 1-11, 1955.

(101) Tuite, J. F., and C. M. Christensen.
Grain storage studies. XXIII. Time of invasion of wheat seed by various species of Aspergillus responsible for deterioration of stored grain and, source of inoculum of these fungi. Phytopothology 47: 265-268, 1957.

(102) Turesson, G.
Über den Zusammenhang zwischen Oxydationenzymen und Keimfähigkeit in verschiedenen Samenarten. Bot. not. 323-335, 1922.

(103) Umbreit, W. W., R. H. Burris, and J. F. Stauffer.
Manometric Techniques (3rd ed.) Burgess Publ. Co., Minneapolis, Minn. 1957.

(104) Warburg, O., H. Klotsch, and G. Krippahl.
Glutaminsäure-decarboxylase in Chlorella. Naturwiss. 44: 235 only, 1957.

(105) Warburg, O., and G. Krippahl.
Beweis der Notwendigkeit der Glutaminsäure für die Photosynthese. Z. für Naturforsch. 13b: 63-65, 1958.

(106) Wingo, J. G., and J. Awapara.
Decarboxylation of glutamic acid in brain. Jour. Biol. Chem. 187: 267-271, 1950.

(107) Zeleny, L., and D. A. Coleman.
Acidity in cereals and cereal products, its determination and significance. Cereal Chem. 15: 580-595, 1938.

(108) Zeleny, L.
Chemical and nutritive changes in stored grain. Cereal Chem. 6: 112-125, 1948.

RELATION OF VIABILITY TO GLUTAMIC AND PYRUVIC DECARBOXYLASES IN WHEAT (TRITICUM VULGARE)

by

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ABSTRACT

Introduction. The importance of cereal grains in human nutrition has been due to a large extent to their good keeping qualities when stored under ideal conditions. However, unfavorable weather during harvesting and improper storage initiate various deteriorative processes in the grain. All these deteriorative changes lower the value of cereal grain both for milling processing and its use for seed.

As pointed out in many earlier papers, humidity and temperature are the most important environmental factors affecting the viability and storage behavior of cereal grain. In the present study various factors involved in deterioration of wheat grains have been investigated with special attention to biochemical methods in determining the degree of viability. The relation of the type of damage to the particular method involved was studied with special emphasis on the relation of "sick" wheat to the application of glutamic and pyruvic acid decarboxylases in viability determinations.

Procedures. The moisture content was determined by the one stage air-oven method, drying the ground samples for one hour at 130°C. "Sick" wheat was determined by carefully removing the pericap covering the germ. All embryos with tan or dark color were considered "sick".

Germination tests were performed on moist sterile quartz-sand in petri dishes, using 100 kernels for each determination. Tests were run in duplicate. All kernels which showed normal sprouts after seven days were considered as viable.

Glutamic and pyruvic acid decarboxylase activities were determined by Warburg manometric techniques. Usually 500 mg of ground wheat grains, or 100 mg of ground wheat germ, were used in the reaction chamber. The flasks were immersed in a water bath of 30.0° ± 0.1°C. Carbon dioxide evolution during 30

minutes was calculated.

A slightly modified procedure of Lakon was used for the topographical tetrazolium (TTC)-test. One hundred half-kernels presoaked in water were treated in 1 percent 2,3,5-triphenyl tetrazolium chloride solution for 6 hours. The colorimetric TTC-test was a modified method of Sorger-Domenigg, et al. One thousand mg of ground whole kernels or 250 mg of ground germ, respectively, were digested in 5 ml of 1 percent TTC-solution in an air-oven at 38°C for exactly one hour. After extraction overnight by acetone the mixtures were filtered, and the absorbance of the extracts was measured with the Beckman Model DU spectrophotometer at 520 mu.

Fluorescence was determined according to a slightly modified method of Cole and Milner. One thousand mg of ground whole wheat or 250 mg of germ were weighed into erlenmeyer flasks containing 25 ml of 0.2 M hydrochloric acid. The mixtures were shaken by hand at certain time intervals and allowed to stand overnight. After filtering the filtrate was diluted if necessary and used as such for fluorescence determination. Measurements were made with Coleman Photoelectric Fluorometer, using B_1-S and PC-1 filters.

Fat acidity was determined according to AACC, Cereal Laboratory Methods. The ground samples were extracted in a Goldfish extractor with 50 ml of Skelly Solve for 6 hours. After removal of the solvent, the extracts were dissolved in 25 ml of isopropylalcohol-benzene mixture containing 0.2 percent phenolphthalein, and titrated with 0.0129 N KOH to a distinct pink color. Fat acidity was reported as mg of KOH required to neutralize the free fatty acids in 100 gram of ground wheat.

Twenty-five of the samples were of old germ-damaged wheat (series 1) which had been stored at +4°C; 20 were "new" wheats from 1959 (series 2); and 4 were samples from the 1959 crop containing frost damaged kernels.

Results. Forty-nine wheat samples were investigated. The series 1 covered reasonably well the germination range from 0 to 100 percent, with a few exceptions. Unfortunately this was not so with the series 2, in which all germination percentages, except for one sample, fell in the area from 77 to 99 percent. The germination of frost damaged samples varied from 74 to 96 percent.

The replicability of Warburg measurements was determined by calculating the intraclass correlation coefficients from 25 samples, each run in triplicate. With whole kernels, $r = 0.984^{***}$, and with germ ends, $r = 0.978^{***}$ were found. The correlations between the different tests performed were evaluated by calculating simple linear correlation coefficients. Standard deviation was calculated by the method of the least squares.

In germ-damaged wheat samples a significantly high negative correlation was found between the amount of "sick" wheat and germination percentage ($r = -0.969^{***}$) or topographical TTC-test ($r = -0.973^{***}$). Correlation coefficients between "sick" wheat and Warburg determinations ($r = -0.878^{***}$ with whole kernels) were also somewhat higher than those involving fluorescence and fat acidity, respectively. It has been known for some time that germ-damage in wheat is followed by high fat acidity. Fat acidity values in series 1 varied from 19.46 to 45.20, and it was found that fat acidity has a highly negative correlation with germination percentage ($r = -0.906^{***}$) and with Warburg measurements ($r = -0.874^{***}$ in whole kernels). The correlation found in the present work between fat acidity and "sick" wheat percentage was only slightly lower than that involving viability. The results from this study as compared to those previously obtained suggested that increased fat acidity is an indication of lowered viability, though the increase in free fatty acids does not have to be primarily associated with any of the known deteriorative processes involved.

Previous work in this laboratory has shown that extracts of "sick" wheat

embryos exhibit an increase in fluorescence, which precedes the respiratory increase indicative of mold growth. On the other hand fluorescence of sound wheat samples of a widely different source, variety, and class has been found low and virtually uniform. In the present work the fluorescence value was determined both with whole ground kernels and with ground germ ends. The correlation between these two measurements was very high ($r = 0.920^{***}$). With a few exceptions there was little change in fluorescence in samples of 100 to 20 percent of germination. The correlation between fluorescence and germination percentage in all of the 25 samples of series 1 was -0.758^{***} (ground whole kernels). However, when correlation coefficients were calculated, neglecting four extreme samples of very high amount of "sick" wheat, poor correlation was obtained ($r = -0.479^{**}$). Similar results were obtained with samples of series 2. The results suggested that correlation between fluorescence and viability, or "sick" wheat actually is curvlinear. At very early stages of deterioration this is nearly linear. When deterioration advances distribution of fluorescence obtained with wheat samples of the same viability progressively increases. In particular, unexpectedly high values of fluorescence were encountered in samples of little or no viability, supporting the theory that primary browning products in "sick" wheat development are not fluorescent; viability may be lost before any browning of germ can be noticed, and visually detectable browning precedes any marked increase in fluorescence. After viability is lost, nonenzymatic browning advances leading to highly fluorescent compounds.

Relatively large variation was found in decarboxylation of glutamate in different samples. Though the simple linear correlations between Warburg tests and viability (series 1: $r = 0.921^{***}$ for whole kernels; $r = 0.933^{***}$ for germ ends), or "sick" wheat ($r = -0.878^{***}$ for whole kernels; $r = -0.882^{***}$ for germ ends) are high and significant, it appeared likely that correlations involving

"sick" wheat actually may be closer to curvlinear. Enzyme activity decreases most rapidly at the early stages of deterioration, when only a few grains have totally lost their viability.

Though particularly the topographical tetrazolium test has been widely accepted as a means to detect viability of seeds, the results have been reported to differ with the variety of wheat, location of growth, and the degree of mold infestation. In the present study the topographical TTC-test generally gave the best correlation with both germination percentage and the amount of "sick" wheat. It was interesting to notice that a high positive correlation exists between the topographical TTC-test and the Warburg test with whole kernels ($r = 0.912^{***}$). The correlation between colorimetric TTC-test and germination percentage was somewhat lower, though highly significant, and it was suggested that particularly in the case of germ ends, this correlation may be curvlinear. At very low viability, and advanced deterioration, reducing compounds formed may possibly contribute to formazan development.

With wheats of the 1959 crop, fat acidity and fluorescence showed a very high negative correlation with germination percentage ($r = -0.920^{***}$, and $r = -0.957^{***}$, respectively). In this series, correlation between decarboxylation of glutamate and germination percentage was rather low ($r = 0.774^{***}$). It has been shown previously that significant differences in the activity of glutamic acid decarboxylase occur in different varieties of wheat, and in wheats from different locations. This led to the conclusion that though the Warburg test with glutamate seemed to have little value in examining "new" wheat samples, it either alone or together with the germination test and determination of the free amino acids may give an excellent picture of the storage background of wheat. Preliminary results with carboxylase activity using pyruvate as a substrate in Warburg measurements indicated a high correlation with viability

in germ-damaged wheat; with new wheat samples further research is necessary.

Germination percentage was found to decrease, and "sick" wheat, fat acidity, and fluorescence to increase with an increasing relative amount of frost damage. This is somewhat contrary to earlier observations, which indicated that fat acidity is low regardless of the degree and amount of frost damage. Hence it has been suggested that freezing of wheat does not cause hydrolytic deterioration of fats. However, it seems quite likely that in prolonged storage, deteriorative processes may be even faster in grains initially affected by frost. The positive correlation between topographical TTC-test and germination percentage was high ($r = 0.996^{***}$). No significant correlation between decarboxylation of glutamate and the germination test was found, possibly due to the small number of samples employed.